Carbonyls in Oxidizing Fat. V. The Composition of Neutral Volatile Monocarbonyl Compounds from Autoxidized Oleate, Linoleate, Linolenate Esters, and Fats

The steam volatile monocarbonyl compounds in mildly autoxidized esters of oleic, linoleic, linolenic acids, and animal and vegetable fats were quantitatively estimated. The major aldehydes in oleate and linoleate were those that might be expected from the scission of reported monomeric hydroperoxide isomers. The predominance of hept-2,4-dienal and propanal in linolenate suggested that the major monomeric hydroperoxides were 12- and 16-hydroperoxy conjugated dienoic isomers. The number of minor aldehydes increased with degree of unsaturation of the fatty acid. The amounts of monocarbonyl compounds in the fats examined generally agreed with their average fatty acid composition. Appreciable amounts of heptanal in lamb and beef fat and heptanal and decanal in butterfat, under the conditions of oxidation, could not have come from the three unsaturated acids. Heating at 165°C. in all samples increased the proportions of the most unsaturated major aldehydes.

OXIDATIVE RANCIDITY in various fats develops at different peroxide levels. This, regardless of causative factors, is a reflection of the kinds and amounts of carbonyl compounds generated and is of great interest in the study of the nature of rancidity. The qualitative analysis of the volatile monocarbonyl compounds produced by mildly oxidized esters of oleic, linoleic, linolenic acids, and several animal and vegetable fats was recently described (5). This report contains an extension to the quantitative estimation of the separated 2,4-dinitrophenylhydrazone derivatives.

Experimental

Materials (except for butterfat [1]), as well as methods, were the same as described in the preceding publication (5), with thin films of samples oxidized under ultraviolet light at room temperature. Hydrazones of steam volatile monocarbonyls from unheated and heated (165°C. for 15 min. in open vessel) samples (10) were separated by paper chromatographic methods into individual compounds (3,11), and these were identified and estimated by previously reported methods and applications (4,11,12,13). Results are based on contents of 10 g. of oxidized sample, with correction factors applied as indicated by experiments with known mixtures (4).

Results and Discussion

Volatile aldehydes measured in oxidized methyl oleate and triolein are shown in Table I. Over-all there was good agreement between the ester and triglyceride. Oleate has been found to form monomeric hydroperoxide isomers 8, 9, 10, and 11 in equal amounts (19). The expected aldehydes from these hydroperoxides, undec-2-enal, dec-2-enal, n-nonanal, and n-octanal, respectively, were the major compounds, but they were not individually equal. n-Nononal predomi-

[1] Furnished by D.P. Schwartz of our Dairy Products Laboratory.

nated in the unheated and undec-2-enal and dec-2-enal in the heated. Since heating at 165°C. was probably accompanied by some loss of the more volatile compounds and further oxidation, the results were not entirely representative of the effect of heat on the hydroperoxides and carbonyl generation. Nevertheless there appeared to be a considerable difference in the scission of monomeric hydroperoxide isomers if they were present and formed in equal amounts.

Ethanal, n-hexanal, n-undecenal, and non-2-enal were also present in minor amounts. Ethanal and non-2-enal were found in both linoleate and linolenate (Table II). These compounds cannot be due to linolenate impurity since the characteristic major monocarbonyl compounds of that acid were not present. Possibly these compounds arise from mutually-contained impurities. n-Hexanal is strongly characteristic of linoleate (Table II). The relatively high proportions of this compound in aldehydes from both oleates make it difficult to explain on the basis of linoleate impurity. However the effect of linoleate impurity would be marked because of its higher oxidation rate. Both oleate samples contained some linoleate impurity as indicated by the detection of traces of alk-2,4-dienals in more oxidized samples. Fritsch and Deatherage (7) found C_6, C_7, C_8, and C_9 saturated acids in decomposed volatile peroxides isolated from methyl oleate oxidized at 80°C. These volatile peroxides yielded carbonyl compounds on reduction and could be partially converted into carbonyl derivatives. The carbonyl compounds which these interesting compounds might yield were not determined (7). n-Undecanal was found only in the oxidized oleates. The mechanism of formation of this compound is not known since the evidence is that the double bond migrates only between the 11 and 8 carbons.

The carbonyl compounds determined in oxidized ethyl linoleate and methyl linoleate are indicated in Table II. According to existing theories, as reviewed by Badings (1), the formation of three isomeric hydroperoxides is possible in autoxidized linoleate. These are the nonconjugated 11 and conjugated 9 and 13 isomers which would yield oct-2-enal, dec-2,4-dienal, and n-hexanal, respectively. Badings (1) found these carbonyls to be the major monocarbonyl compounds in oxidized ammonium linoleate. In this work n-hexanal and dec-2,4-dienal were the predominant volatile aldehydes found. This agrees with the reports (1) that the greater part of the hydroperoxides formed have conjugated double bonds. Hept-2-enal, oct-2-enal, and non-2-enal were present among the minor carbonyl compounds with hept-2-enal predominating in this group. These carbonyl compounds might arise from nonconjugated hydroperoxides, but the lack of supporting evidence for the presence of such hydro-

TABLE I

Comparison of Volatile Monocarbonyl Composition of Ester and Triglyceride

	Monocarbonyl 2,4-Dinitrophenylhydrazones/10 g.							
	Methyl oleate (peroxide 40.8)				Triolein (peroxide 43.0)			
	Unheated		Heated		Unheated		Heated	
	Micromoles	%	Micromoles	%	Micromoles	%	Micromoles	%
Alkanals	4.44	58.2	10.68	29.9	3.34	67.2	8.25	27.4
C_1	....	....	....	....	....	....	....	....
C_2	....	....	....	....	0.42	8.5	....	....
C_3	....	....	....	....	....	....	....	....
C_4	....	....	....	....	....	....	....	....
C_5	....	....	....	....	....	....	....	....
C_6	1.22	16.0	1.45	4.1	0.84	16.9	1.06	3.5
C_7	....	....	....	....	....	....	....	....
C_8	0.79	10.4	1.88	5.3	0.54	10.9	1.04	3.5
C_9	1.85	24.2	5.45	15.2	1.14	22.9	4.40	14.6
C_{10}	....	....	....	....	....	....	....	....
C_{11}	0.58	7.6	1.90	5.3	0.40	8.0	1.75	5.8
Enals	3.19	41.8	25.04	70.1	1.63	32.8	21.87	72.6
C_3	....	....	....	....	....	....	....	....
C_4	....	....	....	....	....	....	....	....
C_5	....	....	....	....	....	....	....	....
C_6	....	....	....	....	....	....	....	....
C_7	....	....	....	....	....	....	....	....
C_8	....	....	....	....	....	....	....	....
C_9	0.45	5.9	1.45	4.1	0.31	6.2	0.84	2.8
C_{10}	1.36	17.8	10.29	28.8	0.73	14.7	9.42	31.3
C_{11}	1.38	18.1	13.30	37.2	0.59	11.9	11.61	38.5

peroxides makes this mechanism of formation questionable (1). Patton *et al.* (18) have reported the presence of hept-2-enal, non-2-enal, dec-2-enal, and dec-2,4-dienal in the steam distillate of methyl linoleate. Modest quantities of ethanal, propanal, and non-2,4-dienal found potentially could come from linolenate impurity, but the major monocarbonyl component of linolenate, hept-2,4-dienal, was not detected. In higher oxidized samples of linoleate, small amounts of dodec-2,4-dienal were found. Chang and Kummerow (2) obtained ethanal, pentanal, and hexanal from oxidized polymers of ethyl linoleate, but pentanal was not found in our study. The small amounts of nonanal detected could not have come from oleate impurity as dec-2-enal and undec-2-enal were not present.

The nature of the process of linolenate autoxidation has not been completely established (16). Fugger

et al. (8) found diene and triene conjugation and only a small amount of monomeric hydroperoxide. As shown in Table II, the major carbonyl compounds were propanal and hept-2,4-dienal. These compounds could come from a single resonating radical form (6), giving diene conjugated hydroperoxides 16 and 12, with unsaturation in 9, 12, 14 and 9, 13, 15 positions, respectively. Heating had the effect of making hept-2,4-dienal and non-2,4-dienal the predominant compounds. In addition to the major carbonyl compounds butanal and but-2-enal, pent-2-enal, and hex-2-enal appeared characteristic of linolenate. However the other minor carbonyl compounds could not be logically traced to linoleate impurity. Kawahara *et al.* (15) found ethanal, propanal, but-2-enal, and pent-2-enal in autoxidized linolenate. Their presence in soybean oil was rationalized by Kawahara and

TABLE II

Volatile Monocarbonyl Content of Oxidized Polyene Esters

	Monocarbonyl 2,4-Dinitrophenylhydrazones/10 g.							
	Ethyl linoleate—pv 200				Methyl linolenate—pv 93			
	Unheated		Heated		Unheated		Heated	
	Micromoles	%	Micromoles	%	Micromoles	%	Micromoles	%
Alkanals	9.84	57.5	12.69	11.6	3.72	45.6	3.70	13.3
C_1	....	....	....	....	....	....	....	....
C_2	1.01	5.9	....	....	0.10	1.2	0.26	0.9
C_3	0.44	2.6	....	....	2.59	31.8	2.65	9.5
C_4	....	....	....	....	1.03	12.6	0.79	2.9
C_5	....	....	....	....	....	....	....	....
C_6	8.39	49.0	9.54	8.7	....	....	....	....
C_7	....	....	....	....	....	....	....	....
C_8	....	....	....	....	....	....	....	....
C_9	....	....	3.15	2.9	....	....	....	....
Enals	3.21	18.7	17.82	16.3	1.94	23.8	4.07	14.6
C_3	....	....	....	....	....	....	....	....
C_4	....	....	....	....	0.27	3.3	0.51	1.8
C_5	....	....	....	....	0.60	7.3	1.49	5.4
C_6	....	....	....	....	0.54	6.6	0.95	3.4
C_7	1.95	11.4	6.96	6.4	0.38	4.7	0.66	2.4
C_8	0.79	4.6	4.00	3.7	....	....	....	....
C_9	0.47	2.7	6.86	6.2	0.15	1.9	0.46	1.6
C_{10}	....	....	....	....	....	....	....	....
C_{11}	....	....	....	....	....	....	....	....
Dienals	4.08	23.8	79.05	72.1	2.50	30.6	20.06	72.1
C_7	....	....	....	....	2.35	28.8	12.56	45.1
C_8	....	....	....	....	....	....	....	27.0
C_9	0.41	2.4	....	....	0.15	1.8	7.50	....
C_{10}	3.67	21.4	79.05	72.1	....	....	....	....
C_{11}	....	....	....	....	....	....	....	....
C_{12}	....	....	....	....	....	....	....	....

Dutton (14) by the hydroperoxide hypothesis of Farmer et al. (6). A small amount of trienal monocarbonyl compound was detected in our work (5), which has not been identified.

Each autoxidized fatty acid produced several major characteristic aldehydes. In the case of oleate and linoleate, these compounds would result from the scission of the monomeric hydroperoxides known to be present. Little is known about the monomeric hydroperoxide isomers of linolenate, but their behavior in carbonyl formation may be similar to the less unsaturated acids. The number of minor monocarbonyl compounds increased with the degree of unsaturation. The mechanism of their formation is not clear. Some may be formed by side reactions involving the volatile peroxides described by Fritsch and Deatherage (7).

The effect of simultaneously oxidizing mixtures of palmitic, stearic, oleic, and linoleic esters is shown in Table III. Mixtures were made up in the proportions usually found in pork, lamb, and beef fat [pork: palmitate 30.1%, stearate 16.2%, oleate 46.6%, and linoleate 7.1%; lamb: 24.4%, 34.5%, 39.1%, and 2.0%, respectively; and beef: 32.0%, 21.0%, 45.9%, and 1.1%, respectively]. Proportions of volatile monocarbonyl compounds found were generally as might be expected from the amounts of oleate and linoleate.

In Table IV a comparison was made of pork, lamb, beef, and butterfat with palm oil (fruit-coat) and cocoa butter. Pork fat and palm oil, and lamb fat cocoa butter are, in general, similar in composition but different in glyceride structure (5). These fats varied considerably in the effect of heating at 165°C.

on the amounts and proportions of monocarbonyl compounds. It is not clear whether this was due to anti-

TABLE III
Comparison of Mixtures of Stearic, Palmitic, Oleic, and Linoleic Esters

	Volatile Monocarbonyl 2,4-Dinitrophenylhydrazones/10 g.					
	Pork mixture (Peroxide 28.0)		Lamb mixture (Peroxide 31.0)		Beef mixture (Peroxide 33.0)	
	Unheated	Heated	Unheated	Heated	Unheated	Heated
	% of 4.40 µm	% of 10.63 µm	% of 5.95 µm	% of 12.36 µm	% of 3.93 µm	% of 15.49 µm
Alkanals	67.1	39.0	63.2	51.1	62.3	43.6
C_2	6.2	......	4.7	......	......	......
C_3	3.2	......	......	......	......	......
C_4	......	......	......	......	......	......
C_5	......	......	......	......	......	......
C_6	28.4	16.2	18.7	12.5	18.6	8.0
C_7	......	......	......	......	......	......
C_8	8.2	4.6	11.8	6.4	10.7	6.9
C_9	16.1	14.2	22.5	27.5	29.5	21.5
C_{10}	......	......	......	......	......	......
C_{11}	5.0	4.0	5.5	5.1	3.5	7.2
Enals	23.6	36.7	29.6	38.3	30.3	47.6
C_3	......	......	......	......	......	......
C_4	......	......	......	......	......	......
C_5	......	......	......	......	......	......
C_6	......	......	......	......	......	......
C_7	2.7	9.5	2.9	4.3	2.3	3.5
C_8	3.8	4.1	2.7	2.6	2.0	2.7
C_9	2.5	2.9	3.9	3.7	4.1	4.4
C_{10}	8.0	11.5	9.9	15.9	12.2	20.7
C_{11}	6.6	8.7	10.2	11.8	9.7	16.3
Dienals	9.3	24.3	7.2	10.2	7.4	8.7
C_7	......	......	......	......	......	......
C_8	......	......	......	......	......	......
C_9	1.4	0.9	1.2	1.3	1.3	0.8
C_{10}	7.0	21.3	4.8	7.5	5.1	6.4
C_{11}	......	......	......	......	......	......
C_{12}	0.9	2.1	1.2	1.4	1.0	1.5

TABLE IV
Comparison of Animal and Vegetable Fats

	Volatile Monocarbonyl 2,4-Dinitrophenylhydrazones/10 g.											
	Pork fat (rendered) (Peroxide 30.8)		Palm oil (Peroxide 27.0)		Lamb fat (rendered) (Peroxide 30.0)		Cocoa butter (Peroxide 33.0)		Beef fat (rendered) (Peroxide 31.5)		Butterfat (Peroxide 30.1)	
	Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated
	% of 3.51 µm	% of 9.70 µm	% of 2.64 µm	% of 5.86 µm	% of 4.79 µm	% of 10.35 µm	% of 3.02 µm	% of 5.52 µm	% of 4.75 µm	% of 7.38 µm	% of 5.61 µm	% of 14.03 µm
Alkanals	65.0	37.9	73.5	58.4	55.5	54.1	66.6	56.2	63.2	49.5	66.3	39.1
C_1	......	......	......	......	......	......	......	......	......	......	1.0	......
C_2	1.7	2.8	4.6	......	2.1	3.2	3.0	......	1.3	......	3.4	......
C_3	11.7	2.4	3.4	......	6.4	7.7	4.6	......	3.4	......	13.0 [a]	......
C_4	......	......	......	......	......	......	......	......	......	......	......	......
C_5	......	......	......	......	......	......	......	......	......	......	8.0	4.6
C_6	27.3	13.9	37.1	31.2	9.8	9.5	24.5	24.1	16.2	10.6	11.1	8.7
C_7	......	......	......	......	11.9	11.0	......	......	7.4	6.4	8.9	8.1
C_8	8.0	4.7	6.1	4.6	6.7	5.7	8.3	6.5	9.1	7.7	5.9	4.4
C_9	12.0	10.7	17.8	18.3	14.4	12.8	23.2	22.3	19.8	18.6	11.1	9.5
C_{10}	......	......	......	......	......	......	......	......	......	......	3.9	3.8
C_{11}	4.3	3.4	4.5	4.3	4.2	4.2	3.0	3.3	6.1	6.2	......	......
Enals	22.5	25.5	15.5	27.7	39.7	37.1	21.9	33.0	29.1	41.6	23.5	30.1
C_3	......	......	......	......	......	......	......	......	......	......	1.8	......
C_4	......	......	......	......	......	......	......	......	1.7	......	2.3	1.7
C_5	2.3	......	1.1	......	2.7	3.6	......	......	......	......	2.5	2.3
C_6	......	......	1.5	......	......	......	......	......	......	......	2.6	3.9
C_7	5.4	5.4	3.8	11.8	3.6	4.1	4.7	10.1	3.0	4.5	2.6	3.2
C_8	4.6	3.5	2.7	4.5	4.4	3.8	3.6	4.0	3.6	4.9	6.1	9.3
C_9	3.4	3.8	3.0	3.9	18.6	12.4	3.0	2.9	8.6	8.2	2.7	4.9
C_{10}	4.0	6.5	2.3	4.8	5.6	7.2	7.0	10.0	6.5	12.6	2.9	4.8
C_{11}	2.8	6.3	1.1	2.7	4.8	6.0	3.6	6.0	5.7	11.4	......	......
Dienals	12.5	36.6	11.0	14.0	4.8	8.8	11.6	10.9	7.8	8.9	10.2	13.0
C_7	4.3	2.8	1.9	2.4	1.7	2.4	2.7	1.8	1.5	1.2	3.4	4.6
C_8	......	......	......	......	......	......	......	......	......	......	......	......
C_9	2.0	1.7	2.7	2.1	1.0	1.3	2.7	1.6	1.7	1.1	2.1	1.6
C_{10}	6.2	29.6	6.4	8.5	2.1	4.1	6.2	6.2	3.6	5.3	2.5	5.5
C_{11}	......	......	......	......	......	......	......	......	Tr.	Tr.	2.2	1.3
C_{12}	......	2.5	......	1.0	......	1.0	......	1.3	1.0	1.3	......	......
2-Alkanone												17.9
C_5	......	......	......	......	......	......	......	......	......	......	......	2.9
C_7	......	......	......	......	......	......	......	......	......	......	......	4.6
C_9	......	......	......	......	......	......	......	......	......	......	......	3.2
C_{11}	......	......	......	......	......	......	......	......	......	......	......	3.4
C_{13}	......	......	......	......	......	......	......	......	......	......	......	2.3
C_{15}	......	......	......	......	......	......	......	......	......	......	......	1.5

[a] Mixture of 2-alkanone C_3 and alkanal C_3.

TABLE V
Volatile Monocarbonyl Composition of Highly
Unsaturated Vegetable Oils

	Carbonyl 2,4-Dinitrophenylhydrazones/10 g.					
	Peanut oil (Peroxide 26.0)		Soybean oil (Peroxide 33.0)		Linseed oil (Peroxide 33.0)	
	Unheated	Heated	Unheated	Heated	Unheated	Heated
	% of 2.22 μm	% of 5.85 μm	% of 3.51 μm	% of 7.70 μm	% of 10.56 μm	% of 16.19 μm
Alkanals	58.6	46.0	57.0	44.6	48.4	32.0
C_1	......	......	1.7	......	......	......
C_2	3.6	......	3.5	......	4.3	1.6
C_3	......	......	13.7	5.5	14.7	12.1
C_4	......	......	......	......	6.0	4.0
C_5	......	......	......	......	......	......
C_6	35.6	32.5	25.4	21.3	12.3	9.4
C_7	......	......	......	......	......	......
C_8	9.5	......	6.2	7.5	......	......
C_9	9.9	13.5	6.5	10.3	11.1	4.9
C_{10}	......	......	......	......	......	......
C_{11}	......	......	......	......	......	......
Enals	23.0	23.4	19.4	25.6	20.1	21.4
C_3	......	......	......	......	......	......
C_4	......	......	......	......	2.3	1.4
C_5	......	......	2.3	......	5.9	5.9
C_6	......	......	2.6	1.6	5.4	5.7
C_7	6.3	12.0	4.3	7.8	4.4	5.3
C_8	6.8	3.7	3.1	3.5	......	......
C_9	7.2	5.1	4.6	7.3	2.1	3.1
C_{10}	1.8	1.2	1.4	3.2	......	......
C_{11}	0.9	1.4	1.1	2.2	......	......
Dienals	18.5	30.6	23.7	29.9	31.5	46.6
C_7	2.7	2.9	10.9	6.2	26.3	27.2
C_8	......	......	......	......	......	......
C_9	3.2	2.1	3.4	2.5	3.2	11.2
C_{10}	12.6	22.4	9.4	20.9	2.0	8.2
C_{11}	......	......	......	......	......	......
C_{12}	......	3.3	......	0.3	......	......

oxidants, glyceride structure, fatty acid content, or to degree of oxidation, as judged from peroxide determination. Peroxide values are not entirely satisfactory as a means of determining comparable degrees of attack by oxygen.

Fairly abundant amounts of heptanal were found in lamb, beef, and butterfat. Butterfat also contained an appreciable amount of decanal. These two carbonyls did not originate from oleate, linoleate, and linolenate. Undec-2,4-dienal was found in small amounts in butterfat but not in the other fats or esters except for traces in beef fat. This is an elusive compound. In earlier work it was found in small amounts in oxi-

dized pork tissue fat (12). Witting and Schweigert (20) found predominant amounts of this compound in lard oxidized with *gamma* irradiation. On the other hand, dodec-2,4-dienal in the current work has been consistently found in linoleate and in most of the fats. The possibility has been considered that undec-2,4-dienal and dodec-2,4-dienal might be mistaken for one another, but this is not believed to be the case (4). It may be that both compounds are present but approach trace amounts in proportion to the other aldehydes (12). Heating the oxidized butterfat produced over 17% 2-alkanone class consisting of C_5, C_7, C_9, C_{11}, C_{13}, and C_{15} ketones. These compounds are not due to oxidation as their formation in heated fresh butterfat has been described by Patton and Tharp (17).

Composition of volatile monocarbonyl compounds found in the more highly unsaturated peanut, soybean, and linseed oils is shown in Table V. Amounts of the major characteristic aldehydes reflect the larger proportions of linoleic and linolenic acids in these oils.

REFERENCES

1. Badings, H.T., J. Am. Oil Chemists' Soc., *36*, 648 (1959).
2. Chang, S.S., and Kummerow, F.A., J. Am. Oil Chemists' Soc., *30*, 251 (1953).
3. Ellis, R., Gaddis, A.M., and Currie, G.T., Anal. Chem., *30*, 475 (1958).
4. Ellis, R., and Gaddis, A.M., Anal. Chem., *31*, 1997 (1959).
5. Ellis, R., Gaddis, A.M., and Currie, G.T., Food Research, in press, (1961).
6. Farmer, E.H., Koch, H.P., and Sutton, D.A., J. Chem. Soc., 1943, 541.
7. Fritsch, C.W., and Deatherage, F.E., J. Am. Oil Chemists' Soc., *33*, 109 (1956).
8. Fugger, J., Cannon, J.A., Zilch, K.T., and Dutton, H.J., J. Am. Oil Chemists' Soc., *28*, 285 (1951).
9. Gaddis, A.M., and Ellis, R., Science, *126*, 745 (1957).
10. Gaddis, A.M., Ellis, R., and Currie, G.T., Food Research, *24*, 283 (1959).
11. Gaddis, A.M., and Ellis, R., Anal. Chem., *31*, 870 (1959).
12. Gaddis, A.M., and Ellis, R., Food Research, *24*, 392 (1959).
13. Gaddis, A.M., Ellis, R., and Currie, G.T., Food Research, *25*, 495 (1960).
14. Kawahara, F.K., and Dutton, H.J., J. Am. Oil Chemists' Soc., *29*, 372 (1952).
15. Kawahara, F.K., Dutton, H.J., and Cowan, J.C., J. Am. Oil Chemists' Soc., *29*, 633 (1952).
16. Morris, S.G., J. Agr. Food Chem., *2*, 126 (1954).
17. Patton, S., and Tharp, B.W., J. Dairy Sci., *42*, 49 (1959).
18. Patton, S., Barnes, I.J., and Evans, L.E., J. Am. Oil Chemists' Soc., *36*, 280 (1959).
19. Privett, O.S., and Nickell, E.C., Fette, Seifen, und Anstrichmittel, *61*, 842 (1959).
20. Witting, L.A., and Schweigert, B.S., J. Am. Oil Chemists' Soc., *35*, 413 (1958).